# METHODS AND COMPOSITIONS USING PEPTIDES AND PROTEINS WITH C-TERMINAL ELEMENTS

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application is a continuation of U.S. application Ser. No. 12/821,050, filed Jun. 22, 2010, which claims benefit of U.S. Provisional Application No. 61/219,086, filed Jun. 22, 2009, and U.S. Provisional Application No. 61/249,140, filed Oct. 6, 2009. Application Ser. No. 12/821,050, filed Jun. 22, 2010, Application No. 61/219,086, filed Jun. 22, 2009, and Application No. 61/249,140, filed Oct. 6, 2009, are hereby incorporated herein by reference in their entirety.

## STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

**[0002]** This invention was made with government support under grants CA104898, CA 119414, CA 119335, CA124427, CA115410, and 30199 from the National Cancer Institute (NCI) of the National Institutes of Health (NIH) and grants W81XWH-08-1-0727 and BC 076050 from the Department of Defense (DoD). The government has certain rights in the invention.

## REFERENCE TO SEQUENCE LISTING

**[0003]** The Sequence Listing submitted Jul. 29, 2019, as a text file named "SBMRI_46.8404_ST25.txt," created on Aug. 7, 2017, and having a size of 63,527 bytes is hereby incorporated by reference pursuant to 37 C.F.R. § 1.52(e)(5).

## FIELD OF THE INVENTION

**[0004]** The present invention relates generally to the fields of molecular medicine, more specifically, to cell and tissue-penetrating peptides.

## BACKGROUND OF THE INVENTION

**[0005]** Peptides that are internalized into cells are commonly referred to as cell-penetrating peptides. There are two main classes of such peptides: hydrophobic and cationic (Zorko and Langel, 2005). The cationic peptides, which are commonly used to introduce nucleic acids, proteins into cells, include the prototypic cell-penetrating peptides (CPP), Tat, and penetratin (Derossi et al., 1998; Meade and Dowdy, 2007). A herpes virus protein, VP22, is capable of both entering and exiting cells and carrying a payload with it (Elliott and O'Hare, 1997; Brewis et al., 2003). A major limitation of these peptides as delivery vehicles is that they are not selective; they enter into all cells. An activatable delivery system can be used which is more specific for one cell type or tissue.

**[0006]** Tissue penetration is a serious limitation in the delivery of compositions to cells. Comparison of the distribution of fluorescein-labeled peptides to that of iron oxide particles coated with the same peptide shows that the particles remain close to the tumor blood vessels, whereas the fluorescent peptide reaches all areas of the tumor. The frequently cited "leakiness" of tumor vessels does not appear to substantially mitigate this problem. Moreover, anti-angiogenic treatments that cause "normalization" of tumor vasculature (Jain, 2005), creating a need to target tumors whose vasculature is not leaky. Thus, it is important to find new ways of improving the passage of diverse compositions into the extravascular space. A number of proteins are known to translocate through the endothelium of blood vessels, including the blood-brain barrier. A prime example is transferrin, which is carried across the blood-brain barrier by the transferrin receptor. This system has been used to bring other payloads into the brain (Li et al., 2002; Fenart and Cecchelli, 2003). Peptide signals for endothelial transcytosis that can mediate translocation of compositions from the circulation into tissues is useful.

**[0007]** Thus, there is a need for new therapeutic strategies for selectively targeting various types of cells, and for internalizing proteins and peptides into those cells and penetration of tissue by proteins and peptides. There is also a need for increasing the delivery of compounds and compositions to and into cells and tissues. The present invention satisfies these needs by providing peptides that can be selectively targeted, and selectively internalized, by various types of cells and/or can penetrate tissue. Related advantages also are provided.

## BRIEF SUMMARY OF THE INVENTION

**[0008]** Disclosed are methods of enhancing internalization, penetration, or both of a co-composition into or through a cell, tissue, or both, the method comprising: exposing the cell, tissue, or both to a CendR element and the co-composition, thereby enhancing internalization, penetration, or both of the co-composition into or through the cell, tissue, or both, wherein, prior to exposing the cell, tissue, or both, the CendR element and the co-composition are not covalently coupled or non-covalently associated with each other.

**[0009]** Also disclosed are methods of enhancing internalization of a co-composition into a cell, the method comprising: exposing the cell to a CendR element and the co-composition, thereby enhancing internalization of the co-composition into the cell, wherein, prior to exposing the cell, the CendR element and the co-composition are not covalently coupled or non-covalently associated with each other.

**[0010]** Disclosed are methods of enhancing penetration of a co-composition into and through a tissue, the method comprising: exposing the tissue to a CendR element and the co-composition, thereby enhancing penetration of the co-composition into and through the tissue, wherein, prior to exposing the tissue, the CendR element and the co-composition are not covalently coupled or non-covalently associated with each other.

**[0011]** Also disclosed are compositions comprising a CendR element and a co-composition, wherein the CendR element and the co-composition are not covalently coupled or non-covalently associated with each other. Also disclosed are compositions comprising a protein or peptide and a co-composition, wherein the protein or peptide comprises a CendR element and an accessory peptide, wherein the CendR element and the co-composition are not covalently coupled or non-covalently associated with each other. Also disclosed are compositions comprising a protein or peptide and a co-composition, wherein the protein or peptide comprises an amino acid sequence, wherein the amino acid sequence comprises a CendR element and an accessory peptide, wherein the CendR element and the co-composition are not covalently coupled or non-covalently associated with each other. Also disclosed are compositions comprising a